ISOLATION AND CHARACTERIZATION OF CHEMICAL CONSTITUENTS FROM THE AERIAL PARTS OF

SPERMACOCELATIFOLIA AUBLET

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ABSTRACT

Spermacocelatifolia (family: Rubiaceae) is one of the important members of the medicinal plants of Bangladesh. Compounds: stigmasterol (1), 2,6-di-t-butyl-4-hydroxymethylene-cyclohexa-2,5-dienone (2), 3β-hydroxy-12-oleanen-28-oic acid (3) and phthalic acid (4) have been isolated from the aerial parts of S. latifolia. Compounds (1), (2) and (3) were isolated from the chloroform extract but compound (4) was isolated from the methanol extract. The structures of the compounds have been established by different spectroscopic data analysis. All four compounds were isolated from this plant for the first time.

KEYWORDS: Spermacocelatifolia, isolation, structure elucidation, spectroscopic methods.

INTRODUCTION

The genus Spermacoce which is belonging to the family Rubiaceae comprises 250-300 species widespread in tropical and subtropical America, Africa, Asia and Europe.¹ Some species in this genus play an important role in the treatment of various diseases as traditional medicine.² Spermacocelatifolia Aublet [Synonym: Borrialatifolia (Aublet) K. Schum.] which is locally known as GhuojhilShak is an important member of the medicinal plants of Bangladesh. The plant is widely distributed in Sri Lanka, India, Bhutan, Malay Peninsula and
tropical Africa. It occurs throughout Bangladesh.\textsuperscript{[3]} It is a prostrate or decumbent annual or perennial herb up to 60 cm tall. It was reported that the root juice of this can be used to treat Malaria.\textsuperscript{[1]} Leaf paste is used by Tanchangya ethnic people in Bangladesh for treatment of boils.\textsuperscript{[3]} Biological investigations such as antimicrobial, cytotoxic and antioxidant activities of methanolic and ethanolic extracts of the plant have been performed and the result showed moderate to strong activity.\textsuperscript{[4-6]} Literature survey showed a few phytochemical studies have been done on this plant so far. In these studies iridoid glycosides, a diterpenoid and pentacyclic triterpene acids have been isolated from this plant.\textsuperscript{1,7-8} Our recent study on the aerial parts of \textit{Spermacoce latifolia} has led to the isolation of stigmasterol (1), 2,6-di-t-butyl-4-hydroxymethylene-cyclohexa-2,5-dienone (2) and 3β-hydroxy-12-oleanen-28-oic acid (3) from the chloroform extract and phthalic acid (4) from the methanol extract (Figure 1). The compounds were isolated for the first time from this plant.

![Chemical Structures](image)

\textbf{Figure 1: Structures of the isolated compounds with numbering.}
MATERIALS AND METHODS
Melting points were determined by thin disc method on a Fisher-John’s electrothermal melting point apparatus. UV spectra were recorded in methanol on a Shimadzu UV-Visible spectrophotometer. IR spectra were recorded on a Shimadzu FT-IR spectrometer as thin film or KBr disc. NMR spectra were recorded in CDCl₃ or CD₃OD or DMSO-d₆ using Bruker WH 400 MHz NMR spectrometer. Mass spectra of the compounds were measured on Finnigan Mat SSQ 710 spectrometer with ionization induced by electron impact at 70 eV. Separation by column chromatography was carried out using silica gel 40 (70-230 mesh, E. Merck). Thin layer chromatography was carried out on TLC plastic sheets pre-coated with silica gel 60 F₂₅₄ (E. Merck).

Collection of the plant materials
The aerial parts of matured *Spermacoce latifolia* plants were collected from Jahangirnagar University campus, Savar, Dhaka. The plant was identified by Bangladesh National Herbarium at Dhaka and a voucher specimen (specimen no. 37755) has been deposited at the herbarium.

Extraction of the aerial parts of *S. latifolia*
The finely ground air-dried plant materials (850 g) were extracted successively with chloroform and methanol at room temperature. At first, the plant materials were soaked in chloroform at room temperature for 72 hours. The extraction solvent was then removed by filtration and fresh distilled solvent was added to the plant materials. The extraction process was repeated for three times and the combined filtrate was evaporated and dried completely by using a rotary evaporator under reduced pressure at a temperature below 45°C to get greenish crude chloroform extract (12.8 g). Then methanol was added to the residual plant materials and the process was repeated as above to get the brownish crude extract (8.0 g).

Isolation of compounds from crude extracts
The crude chloroform extract (12.8 g) was subjected to column chromatography eluted with pet. ether, pet. ether - ethyl acetate and ethyl acetate - methanol in gradient manner. The collections from the column were divided into eleven fractions according to their TLC behaviors. Fraction number 6 (3 g) of the column was rechromatographed by using a medium sized silica gel column eluted with the solvent systems, pet. ether and pet. ether - ethyl acetate successively. Compounds (1, 94 mg) and (2, 78 mg) were isolated from the sub-
fractions 2 and 4 of the column, respectively. To remove the color impurities, fraction number 8 (2.3 g) of the crude chloroform extract was treated with activated charcoal and then rechromatographed by using a silica gel column to get the compound (3, 69 mg) in pure form.

The crude methanol extract (8 g) was triturated with chloroform and ethyl acetate successively and then the residue (4.1 g) was divided into two fractions according to their solubility in methanol at room temperature. The methanol insoluble part (0.6 g) at room temperature was heated in methanol at 60°C and found a clear solution which was stand for overnight and the compound (4, 157 mg) was isolated in pure form as brownish white crystals.

**Spectroscopic data of the isolated compounds**

**Stigmasterol (1)**

White crystal (94 mg); mp 168-170°C; IR (KBr disc) ν 3431 (O-H), 3073, 2936, 2867, 1655 (C=C), 1452, 1362, 1051 cm⁻¹; ¹H NMR (CDCl₃) δ 5.34 (1H, m, H-6), 5.14 (1H, dd, J = 8.4 & 15.2 Hz, H-21), 5.01 (1H, dd, J = 8.4 & 14.8 Hz, H-20), 3.51 (1H, m, H-3), 2.27 (2H, m), 1.94 (2H, m), 1.84 (2H, m), 1.43-1.60 (15H, m), 1.20 (3H, m), 1.12-1.17 (2H, m), 1.04 (3H, s, -CH₃), 0.93 (3H, d, J = 6.4 Hz, -CH₃), 0.83 (3H, t, J = 7.6 Hz, -CH₃), 0.78 (3H, d, J = 6.8 Hz, -CH₃), 0.75 (3H, s, -CH₃), 0.68 (3H, d, J = 6.8 Hz, -CH₃); ¹³C NMR (CDCl₃) δ 140.7 (C-5), 138.3, 129.3, 121.7, 72.1 (C-3), 56.8, 56.7, 50.2, 46.1, 42.5, 42.4, 40.5, 37.8, 36.5, 32.3 (2C), 31.9, 31.6, 28.9, 28.8, 25.4, 24.4, 21.3, 21.2, 20.5, 19.8, 18.7, 12.2, 12.1; MS m/z 412 (M⁺), 397, 394, 382, 351, 314, 273, 255, 231, 213, 81, 67, 55.

**2,6-Di-t-butyl-4-hydroxymethylene-cyclohexa-2,5-dienone (2)**

Yellow oily substance (78 mg); UV (MeOH) λ_max 249, 220 nm; IR (thin film) ν 3418 (O-H), 3094, 2957, 2871, 1690 (C=O), 1656 (C=C), 1456, 1377, 1077 cm⁻¹; ¹H NMR (CDCl₃) δ 6.78 (2H, d, J = 2.4 Hz, H-3 & H-5), 5.60 (1H, br. s, -OH), 5.35 (1H, t, J = 2.4 Hz, =CH-OH), 1.15 (18H, s, 6 -CH₃); ¹³C NMR (CDCl₃) δ 186.0 (C-1), 149.6 (C-2 & C-6), 137.1 (=CHOH), 136.3 (C-3 & C-5), 115.2 (C-4), 31.2 (2C), 26.7 (6 -CH₃); MS m/z 234 (M⁺), 219, 204, 189, 177, 120, 103, 75, 49.

**3β-Hydroxy-12-oleanen-28-oic acid (3)**

White powder (69 mg); mp 306-308°C; IR (KBr disc) ν 3421 (O-H), 2927, 2870, 1691 (C=O), 1653 (C=C), 1458, 1382, 1276, 1033 cm⁻¹; ¹H NMR (CD₃OD) δ 5.22 (1H, t, J = 15.2
Hz, H-12), 3.15 (1H, dd, J = 12.8 & 15.6 Hz, H-3), 1.91-2.10 (3H, m), 1.48-1.65 (5H, m), 1.32-1.38 (10H, m), 1.15-1.28 (7H, m), 1.06 (3H, s, -CH₃), 0.94 (3H, s, -CH₃), 0.91 (3H, s, -CH₃), 0.89 (3H, s, -CH₃), 0.84 (3H, s, -CH₃), 0.81 (3H, s, -CH₃), 0.73 (3H, s, -CH₃); ¹³C NMR (CD₃OD) δ 181.6 (C-28), 139.6 (C-13), 126.9 (C-12), 79.6 (C-3), 56.7, 54.3, 47.6, 43.2, 40.7, 40.4, 40.0, 39.8, 38.1, 34.3, 31.7, 31.6, 30.7, 29.2, 28.7, 27.9, 25.3, 24.3, 24.1, 23.9, 21.5, 19.4, 17.8, 17.6, 16.3, 16.0; MS m/z 456 (M⁺), 438, 426, 248, 203, 189, 175, 147, 133, 119, 105, 95, 81, 69, 43.

**Phthalic acid (4)**

Brownish white crystal (157 mg); mp 230-231°C; UV (CH₃OH) λ max 205 nm; IR (KBr disc) ν3315 (O-H), 3078, 1685 (C=O), 1600 (aromatic C=C), 1384 cm⁻¹; ¹H NMR (DMSO-d₆) δ 8.15 (2H, m, H-3 & H-6), 8.02 (2H, m, H-4 & H-5), ¹³C NMR (DMSO-d₆) δ 172.3 (CO), 125.5, 124.2, 121.3 (C-1 & C-2); MS m/z 166 (M⁺), 148, 121, 104, 76, 52.

**RESULTS AND DISCUSSION**

Successive extraction of dried aerial parts of plant *Spermacocelatifolia* with chloroform and methanol at room temperature yielded 12.8 g and 8.0 g of crude extract, respectively. Fractionation and isolation works on the chloroform by repeated column chromatography have afforded to get stigmasterol (1), 2,6-di-t-butyl-4-hydroxymethylene-cyclohexa-2,5-dienone (2) and 3β-hydroxy-12-oleanen-28-oic acid (3) in pure form (Figure 1). Phthalic acid (4) has been isolated from methanol extract using trituration and crystallization methods. The structures of the compounds were elucidated using spectroscopic techniques.

The mass spectrum of the compound(1) showed a molecular ion peak at m/z 412 which is corresponding to the molecular formula C₂₉H₄₈O. The IR spectrum showed a broad absorption band at 3431 cm⁻¹ indicating the presence of hydroxyl group and absorption bands at 1655& 1051 cm⁻¹ due to C=C and alcoholic C-O stretching vibrations, respectively. The ¹H NMR spectrum showed 3H singlets at δ 1.04 and 0.75; 3H doublets at δ 0.93, 0.78 and 0.68; and 3H triplet at δ 0.83 clearly indicated the presence of six methyl groups in the molecule. The peak at δ 5.34 (1H, m) confirmed the presence of olefinic proton at C-6. The other two olefinic protons at C-20 & C-21 indicated by the two doublet of doublets at δ 5.01 and 5.14. This was further supported by the four peaks in the ¹³C NMR spectral data at δ 140.7, 138.3, 129.3 and 121.7 for four olefinic carbons. The one proton multiplet at δ 3.51 indicated the presence of >CH-OH group in the compound. The carbon, C-3 attached with hydroxyl group
also supported by $^{13}$C NMR spectrum by showing peak at $\delta$ 72.1. The presence of 29 carbons in the molecule of (1) was clearly indicated by the $^{13}$C NMR spectrum. It suggested that compound (1) is a steroidal molecule containing one hydroxyl, two double bonds and six methyl groups. The peaks showed in the mass spectrum can be explained by the fragmentation ions of stigmasterol. Based on all spectroscopic data, literature values and melting point (168-170°C) confirmed that compound (1) is stigmasterol.

Compound (2) was a yellow oily substance. The mass spectrum showed a molecular ion peak at m/z 234 which is corresponding to the molecular formula C$_{15}$H$_{22}$O$_{2}$. The UV absorption of the compound at $\lambda_{\text{max}}$ 220, 249 nm indicated the presence of conjugation and chromophoric groups in the structure. The IR spectrum of the compound showed sharp bands at 1690 and 1656 cm$^{-1}$ suggested the presence of C=O and C=C bonds stretching vibrations, respectively. The presence of hydroxyl group was evident from the broad absorption at 3418 cm$^{-1}$. The two protons doublet at $\delta$ 6.78 and one proton triplet at $\delta$ 5.35 in the $^1$H NMR spectrum indicated the presence of H-3 & H-5 and proton in the system =CHOH, respectively. The coupling constants of these protons showed that two equivalent protons at H-3 & H-5 are coupled with the hydroxymethylene proton due to long range coupling of the conjugated system. The sharp singlet at $\delta$ 1.15 indicated the presence of six equivalent methyl groups in the compound. $^{13}$C NMR spectrum showed seven peaks for fifteen carbons. The carbonyl carbon indicated by the peak at $\delta$ 186.0 and two peaks at $\delta$ 149.6 & 136.3 due to the presence of two pairs of equivalent carbons C-2, C-6 & C-3, C-5, respectively. Six equivalent methyl carbons identified by the most intensified peak at $\delta$ 26.7. The fragment ions present in the mass spectrum at m/z 219, 204, 177, 120, 103, 75 strongly supported the structure of the compound. The above spectral data analysis confirmed that compound (2) is 2,6-di-t-butyl-4-hydroxymethylene-cyclohexa-2,5-dienone.

Compound (3) was a white powder with mp 306-308°C. The mass spectrum showed a molecular ion peak at m/z 456 which is corresponding to the molecular formula C$_{30}$H$_{48}$O$_{3}$. The IR spectrum of the compound showed absorption bands at 3421 and 1691 cm$^{-1}$ indicated the presence of O-H and C=O groups, respectively. The weak band observed at 1653 cm$^{-1}$ indicated C=C stretching vibration. In the $^1$H NMR spectrum, the one proton triplet at $\delta$ 5.22 clearly indicated the presence of an olefinic proton which was supported by peaks at $\delta$ 139.6, 126.9 in the $^{13}$C NMR spectrum. The doublet of doublets at $\delta$ 3.1 was an evidence of -CHOH group in the compound. This was further supported by the $^{13}$C NMR spectral data at $\delta$ 79.6.
The seven methyl groups were represented by seven singlets at the region of $\delta$ 0.73 to 1.06 in the $^1$H NMR spectrum. The presence of carboxyl group was confirmed by the peak at $\delta$ 181.6 in the $^{13}$C NMR spectrum. Finally the presence of 30 carbons in the molecule of compound (3) was clearly indicated by the 30 peaks in the $^{13}$C NMR spectrum which suggested the compound is a triterpenoid containing one hydroxyl, one double bond and one carboxylic acid groups. The important mass peaks at m/z 248 (base peak) and 203 readily explain the presence of pentacyclic triterpenoid skeleton containing a -COOH group in the compound. Based on all spectroscopic data, literature values$^{[10]}$ and melting point of the compound, it was confirmed that compound (3) is 3β-hydroxy-12-oleanen-28-oic acid.

Compound (4) was a brownish white crystal with mp 230-231°C. The mass spectrum of the compound showed a molecular ion peak at m/z 166 which is corresponding to the molecular formula C$_8$H$_6$O$_4$. The UV spectrum showed the absorption band at $\lambda_{max}$ 205 indicated the presence of chromophoric groups in the compound. The IR spectrum of the compound showed a broad absorption band at 3315 cm$^{-1}$ due to the presence of O-H group in the structure. Two bands at 1685 and 1600 cm$^{-1}$ indicated the presence of C=O and aromatic C=C bonds stretching vibrations, respectively. In the $^1$H NMR spectrum, the presence of two multiplets at $\delta$ 8.15 and 8.02 clearly indicated an ortho-disubstituted benzene ring. $^{13}$C NMR spectrum showed four peaks for eight carbons. The C=O of carboxyl groups represented by the peak at $\delta$ 172.3. The fragment ion at m/z 148 (M$^+$-H$_2$O) showed in the mass spectrum also supported in favor of the structure of phthalic acid. Based on all spectroscopic data and melting point$^{[11]}$ of the compound, it was confirmed that the compound (4) is phthalic acid.

**CONCLUSION**

Literature survey showed that very little phytochemical studies have been done on the plant *Spermacoce latifolia*. The isolation and characterization of four compounds from the aerial part of the plant have been reported here. We believe, there is a scope to do more detailed phytochemical and biological study on this plant in future.

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REFERENCES